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Two Prominent Entomopathogenic Fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin as Natural Enemies of Lepidopteran Larvae from Rayalaseema Region of Andhra Pradesh

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Roving survey was conducted for searching the natural occurrence of *Beauveria bassiana* and *Metarhizium anisopliae* during *kharif* season of 2022 and *rabi* season of 2022-23 in Kurnool, Kadapa, Chittoor and Anantapuramu districts of Andhra pradesh. The crops surveyed were Groundnut, Maize, Cotton, greengram and blackgram during September to October of *kharif* season and January to February of *rabi* season. The pest population found in among groundnut crops were *Spodoptera litura*, *Helicoverpa armigera* and *Aproarema modicella* and maize, *Spodoptera frugiperda* which were found at vegetative stage. The fungal infected cadavers of lepidopteran lavae were observed on the soil below the canopy or adhering to the foliage of groundnut and maize crops. The larval cadavers were collected in separate aseptic vials and soil samples were also collected from the surveyed fields. During the survey, it was found that the mean number of infected cadavers with fungi varied from 2.6 to 3.2 per square meter in Kurnool district during Sep-Oct of 2022. In Anantapuramu district the mean number of cadavers were 1.4-2.2 during Jan-Feb of 2023. In Chittoor district the mean number of infected cadavers were varied from 2.4-3.6, whereas in Kadapa district it was 0.2 per square meter during Jan-Feb of 2023. The cadavers were cultured in the lab and based on morphological characters confirmed the isolates as *Beauveria bassiana* and *Metarhizium anispoliae.* The cadavers of *B. bassiana* were found to be high in Kurnool district followed by Chittoor district and least in Kadapa district where as the cadavers of *M.anispoliae* were found to be higher in Chittoor district followed by Anantapuramu district and Nil in Kadapa district.

Keywords: Survey; occurrence; entomopathogenic fungi; Beauveria bassiana; Metarhizium anisopliae.

1. INTRODUCTION

The extensive use of synthetic insecticides led to the development of resistance and resurgence in insect pests in addition to posing problems like pesticide residues and environmental pollution. Eco-friendly pest management techniques have been gaining much attention since 5 to 6 decades. In the line of biological control of insects, microbial pathogens such as bacteria, fungi, viruses, nematodes, and protozoans are promising agents. Among the various microbial agents, entomopathogenic fungi are considered as most important. “Because the fungal pathogens causing the disease to the insects are practically more significant as they are epizootic in nature. Fungi unlike bacteria or viruses, do not require ingestion for infection, so the sucking insects are also targeted either by primary contact or by secondary uptake of the pathogen from sprayed vegetation. Entomopathogenic fungi are natural enemies of diverse terrestrial arthropods and are important regulators of host populations in terrestrial ecosystems” [1]. “They naturally occur in insect hosts as infections; they can be collected in the field and grown in the laboratory for the documentation of the fungus. *Beauveria bassiana* have been reported to occur naturally in more than 700 species of insect hosts” [2]. “There are more than 700 species of fungi from 9 genera that infect insects” [3]. “Entomopathogenic hyphomycetes have great potential as biological control agents against insects and as one component within integrated pest management systems. They are being developed worldwide for the control of many pests of agricultural importance” [4]. “Entomopathogenic fungi are widely distributed in a wide range of habitats including aquatic, forest, agricultural and pasture habitats” [5]. “However, epizootics caused by entomopathogenic fungi in agricultural habitats are more numerous, particularly in temperate regions, than those in other habitats. The occurrence of entomopathogenic fungi in India has been reported by many workers” [6]. These findings suggest that these fungi act as mortality factors in Indian insect populations.

“Beauveria bassiana is the asexual form (anamorph) of *Cordyceps bassiana* fungus, which is the sexual reproducing form (teleomorph). It is an important EPF which

belongs to the class Deuteromycetes. It consists of several species like *B. bassiana*, *B. amorpha*,

A. *brongniartii*, *B. calendonica*, of which *B. bassiana* is widely recognized as an insect pathogen. It causes white muscardine disease in insects like caterpillars, whiteflies, aphids, thrips, grasshoppers, and beetles" (Sinha *et al*., 2016).

"Metarhizium anisopliae is a well-studied soil-inhabiting fungus, having a worldwide distribution ranging from the arctic to the tropics" (Senthil-Nathan, 2015). "It is commonly referred as the green muscardine fungus. The susceptible insect hosts of *Metarhizium* include beetles [coleopteran order, primarily scarabidae family], root weevils, flies, thrips, gnats etc" [7]. "The commercial and indigenous strains of *Metarhizium* fungus were found to be effective against white grubs" [8]. Also, the pod borer (*Helicoverpa armigera*) in chickpeas was effectively controlled by using *M. anisopliae* in a study conducted by [9].

The occurrence of entomopathogenic fungi in India has been reported by many workers. However, the abundance and biodiversity of these fungi is less known, which can be explored and documented with the help of authentic and accurate techniques, therefore a survey was carried out in various places of Rayalaseema zone in Andhra Pradesh to isolate and identify the indigenous fungal isolates.

2. MATERIALS AND METHODS

2.1 Survey for Collection of Test Fungi

A Roving survey was conducted for the collection of cadavers for the isolation of *Beauveria bassiana* and *Metarhizium anisopliae* on insect pests in field crops of four Groundnut growing districts in Rayalaseema region of Andhra Pradesh. The survey was conducted two times i.e during September-October, *kharif* 2022 and January-February, *rabi* 2022-2023. The districts selected in Rayalaseema region of Andhra Pradesh were Kurnool, Kadapa, Chittoor and Anantapuramu. In the selected districts, two Mandals were selected and two villages were selected from each mandal. From Kurnool district, Pathikonda (Chakaralla and Pothiralla village) and Devanakonda (karivemula and Kapatralla village) mandals were selected. From Anantapuramu district, Gooty (Thondavada and ubicherla village) and Bukkaraya samudram (Reddipalle and Rekulakunta village) mandals were selected. From Chittoor district, Gangavaram (Dandapalle and Gangavaram village) and palamaner (Gundlapalle and Jagamarla village) mandals were selected. From Kadapa district, Chintakommadinne (Utukur and Thadiguntla village) and khajipet (Bhumayapalle and Midutur Village) mandals were selected. In each village, the major field crops available in the season were observed for *Beauveria bassiana* and *Metarhizium anisopliae* infected cadavers of insect pests. The crops surveyed were groundnut, Maize, Cotton, Blackgram, Sorghum. Among all the crops observed most of the pest population was observed in Groundnut and maize crops may be due to the good crop canopy that maintains favourable microclimate to EPF. Pests observed during survey were Tobacco caterpillar, *Spodoptera litura*, American boll worm, *Helicoverpa armigera* and Groundnut leaf miner, *Aproarema modicella* in groundnut crop and Fall army worm, *Spodoptera frugiperda* in maize crop.

The soil type observed was mostly black soil in Kurnool and Kadapa districts where as in Anantapuramu district, the soil type was both red soil and black soil and In case of Chittoor district the soil type was sandy loam. The sampling fields were selected randomly in the village. From the fields, the dead cadavers adhered to the leaves, any plant part with external signs of mycosis were collected with the help of fine brush into sterilized glass/plastic vials and plastic petriplates. "After collection of entomopathogenic fungal cadavers into the sterilized vials and the containers were wrapped with parafilm and labelled with the details of date of collection, insect cadaver, crop, village, district and zone. The insect cadavers per square meter were counted at five places randomly in the field" [10].

2.2 Isolation of Test Fungi

From Entomopathogenic fungal cadavers collected during survey, the cadavers was inoculated on to the SMAY medium in Petriplates for confirmation of Entomopathogenic fungi. The cultures were studied morphologically under research microscope and based on the morphological characters they were confirmed as *B. bassiana* and *M. anisopliae.* After confirmation, pure cultures of isolates were and maintained in the laboratory.

3. RESULTS AND DISCUSSION

The pests population observed in Groudnut are *Spodoptera litura,Helicoverpa armigera* and

Aproarema modicella and *Spodoptera frugiperda* in maize crop. The mummified lepidopteran larvae of EPF were observed in fields of Groundnut and maize which were either adhering to leaves or present on soil. Most of the cadavers were found adhering to leaves. Some cadavers were with white fungal mat covered on body except head portion and some covered with light green spore mass. Mostly later instars of *Spodoptera litura, Helicoverpa armigera, Spodoptera frugiperda* and *Aproarema modicella* were seen as cadavers. While collecting the cadavers, white or green spore mass was also observed on leaves or plant parts.

In Chakaralla and Pothiralla village of Kurnool distrsict most of the pest population found was *S. litura* in Chakaralla village and Aproarema modicella in Pothiralla village. most of the cadavers were found to be infected with *Beauveria bassiana* and few were infected with *Metarhizium* Sp.in groundnut crop as the most of the farmers follow groundnut cropping system in these villages. From Anantapuramu district Thondapadu, Tadiguntla and Rekulakunta villages *S. litura* population was found to be higher. The cadavers were found infected with *Metarhizium* Sp. and few were found to be infected with *B. bassiana*. In these villages most of the fields were rainfed and groundnut cropping system is followed. In Chittoor district, Dandapalle and Gangavaram villages, crops found were groundnut and cabbage. The pests observed were *H. armigera* and *S. litura* in groundnut crop. Similarly in Gundlapalle village of Palamaner, higher pest population Of *H. armigera* and *S. litura* was found. Most of the cadavers were found to be infected with *M. anisopliae* and few were found to be infected with *B. bassiana*. From Kadapa district in village utukur village, the groundnut fields were found to be less infested and only *S. litura* was observed. The cadavers found were very less and infection is seen only from *B. bassiana.*

Mostly the cadavers were observed in Groundnut crop maybe due to canopy coverage and buildup of microclimate within the crop field that favours the EPF present in the soil.

Incidence of Entomopathogenic fungi infected insect larvae in different districts of Rayalaseema region (Table 1).

Kurnool district: The pests observed during survey were *Spodoptera litura*, *Helicoverpa armigera* and *Aproarema modicella* in the fields of Groundnut. EPF cadavers of all three caterpillars were found in groundnut crop in Chakaralla, Pothiralla, karivemula and Kapatralla of Kurnool district and mean number of cadavers per square meter during Sep 2022 were 3.2, 2.4, 2.2 and 0.0 respectively. In Kapatralla village no insect cadavers were found, it is mostly may be because of absence of fungal inoculum in the soil as the weather parameters are not favourable in comparision with the other villages. During Sep 2022 the average maximum, minimum temperatures recorded in Kurnool district were 33°C and 22°C. The average morning and evening relative humidities recorded were 37% and 96% respectively. Rainfall of 80 mm was received during the period. During Jan 2023 temperatures recorded were 34°C and 14°C. The average relative humidities recorded were 14% and 89%. Rainfall was not received during the period. Most of the cadavers Collected from this district are found to be infected with *B. Bassiana* and few cadavers were found to be infected with *M. anispolaie*.

Anantapuramu district: During second fortnight of September 2022, most of the pest population observed was *Spodoptera litura.* EPF cadavers of *Spodoptera litura* were recorded in groundnut crop in Tondapadu and Ubicherla villages of Gooty mandal in Anantapuramu district. The mean number of cadavers found were 2.2 and 1.4 per square meter respectively. During the survey period the average maximum, minimum temperatures recorded in Anantapuramu district were 36°C and 22°C. The average morning and evening relative humidities recorded were 37% and 98% respectively. Rainfall of 10 mm was received during the period. During Feb 2023 temperatures recorded were 32°C and 12°C. The average relative humidities recorded were 23% and 96%. Rainfall was not received during the period. Most of the cadavers collected from this district were found to be infected with *M. anisopliae* and few cadavers were infected with *B. bassiana*.

Chittoor district: The pests observed during the survey were *Spodoptera litura* and *Helicoverpa armigera* in Groundnut and *Spodoptera frugiperda* in maize crop. EPF cadavers of *Spodoptera litura* and *Helicoverpa armigera* and *Spodoptera frugiperda* were observed in groundnut at Dandapalle mandal and the mean numbers of cadavers found were 3.6 per square meter and in Gangavaram village of Palamaner manndal the mean number of cadavers found were 2.7 per square meter during the survey

conducted in second fortnight of October, 2022. The average maximum, minimum temperatures recorded in Chittoor district were 30°C and 22°C.

The average morning and evening relative humidities recorded were 51% and 91% respectively. Rainfall of 118.2 mm was received during the period. During Feb 2023 temperatures recorded were 32°C and 16°C. The average relative humidities recorded were 45% and 90%. Rainfall was not received during the period. Most of the cadavers collected from this district were found to be infected with *M. anisopliae* and few cadavers were infected with *B. bassiana.*

Kadapa district: In this District EPF cadavers were found on *Spodoptera litura* and the infected cadavers were seen on groundnut leaves and mean number of cadavers per square meter were 0.2 in Utukur mandal. In other surveyed villages the fields were observed with less pest population, may be due to the use of insecticides. The average maximum, minimum temperatures recorded durimg Oct 2022 in Kadapa district were 30°C and 22°C. The average morning and evening relative humidities recorded were 46% and 75% respectively. Rainfall of 56.78 mm was received during the period. During Feb 2023 temperatures recorded were 29°C and 14°C. The average relative humidities recorded were 40% and 89%. Rainfall was not received during the period. The cadavers found were very less and infection is seen only from *B. bassiana.*

The findings of the present study reveal that considerably the higher density of EPF infected lepidopteran larvae of 3.6 per square meter was found in Dandapalle village of Chittoor district and 3.2 per square meter in Chakaralla village of Kurnool district might be due to higher rainfall received during Sep 2022 with an amount of 80mm in Kurnool district and 118.2mm in Chittoor district that creates good relative humidity of 90-95% and the temperatures are also found to be favourable in these districts. In this zone the usage of synthetic pesticides and fertilizers is very less which creates opportunity for the survival of soil fauna including the soil borne microorganisms like EPF.

The lower incidence of EPF infected lepidopteran larvae of 0.2 per square meter in Kadapa district might be due to lower rainfall of 56mm and relatively less humidity of 40-60% which are unfavourable conditions for infection that generally create conditions that are not suitable for soil microbes like EPF. Usually under these conditions the soil inoculum will be generally low which is the another reason for lower incidence.

The results of present studies were similar to Nidhi et al. [11] conducted a survey in the Kumaun and Garhwal divisions of Uttarakhand for both infected cadavers and soil samples. Isolation of 52 soil samples showed six entomopathogenic fungi. *B. bassiana* infected cadavers were collected among which mycosis was greatly pronounced in *Trichoplusia orichalcea* with *N. rileyi*, followed by *B. bassiana*. Similarly, Dhar et al. [12] isolated 13 fungal isolates, having characteristics similar *to B. bassiana* from infected cadavers and soil samples collected from the fields of different crops in Punjab.

Abarna and Vishnupriya [13] reported that Natural incidence of EPF was high during the month of December. Twelve EPF isolates were isolated from the mycosed *Tetranychus urticae* cadavers and found to be infected with *Beauveria bassiana* and Cocoka et al. [14] surveyed three localities in eastern DR Congo and collected infected cadavers of Fall army worm and earwigs and based on the morphology they confirmed it as *B. bassiana*.

The results are also supported by Manjula et al. [15], who found that 100 per cent Groundnut leaf miner population was mycosed during II FN October and I FN September, 2018. The blotches of leaf miner looked milky white with cadavers protruding from the blotch. The fungus was inoculated and identified as *Metarhizium anisopliae*.

Velavan et al. [16] collected insect cadavers and soil samples from diferent forest types, viz., wet evergreen, moist deciduous, dry deciduous and scrub type in South India and isolated 27 isolates of *Metarhizium anisopliae* which were confirmed based on morphological and molecular studies.

The present results are in line with Ayele et al. [17], who isolated twenty five *Metarhizium* isolates isolated from different soil types, using the great wax moth, *Galleria mellonella* baiting method. Likewise, Elham et al. [18] used the insect baiting approach utilising mealworm larvae, *Tenebrio molitor*, to isolate EPF from soil samples. The only EPF species detected in soil samples from Taman Tropika Kenyir and BRIS soil of Marang was *Metarhizium anisopliae*.

Korosi et al. [19] conducted a survey to isolate and identify the entomopathogenic fungi from soil. Out of 240 soil samples taken from eight different vineyard sites, 144 samples (60%) were positive for the presence of entomopathogenic fungi in the genera of *Beauveria* or *Metarhizium*, with 20 samples (8%) containing both [20,21].

As per the current study the maximum Mean no. of cadavers/m2 area was observed in the month of September and January. This clearly indicates that moderate temperature along with high humidity is necessary for the growth and development of EPF and to cause natural infection to insects [22-23].

Naming of isolates: The isolates were identified based on their morphological characteristics and named based on their area of collection. The spores of *B.bassiana* were round to oval in shape where as the spores of *M. anisopliae* were cylindrical in shape. A total of six isolates of *B.bassiana* (Fig. 1) and six isolates of *M. anisopliae* (Fig. 2) were pure cultured, named and maintained for further research.

Beauveria bassiana isolate Dpl.Ctr
Beauveria bassiana isolate Plmr.Ctr
Beauveria bassiana isolate Pkd.Knl
Beauveria bassiana isolate Dvk.Knl
Beauveria bassiana isolate Gty.Atp
Beauveria bassiana isolate Utkr.Kdp
Metarhizium anisopliae isolate Dpl.Ctr
Metarhizium anisopliae isolate plmr.Ctr
Metarhizium anisopliae isolate Gty.Atp
Metarhizium anisopliae isolate Rkl.Atp
Metarhizium anisopliae isolate Pkd.Knl
Metarhizium anisopliae isolate Dvk.Knl

Fig. 1. Isolates of *Beauveria bassiana*

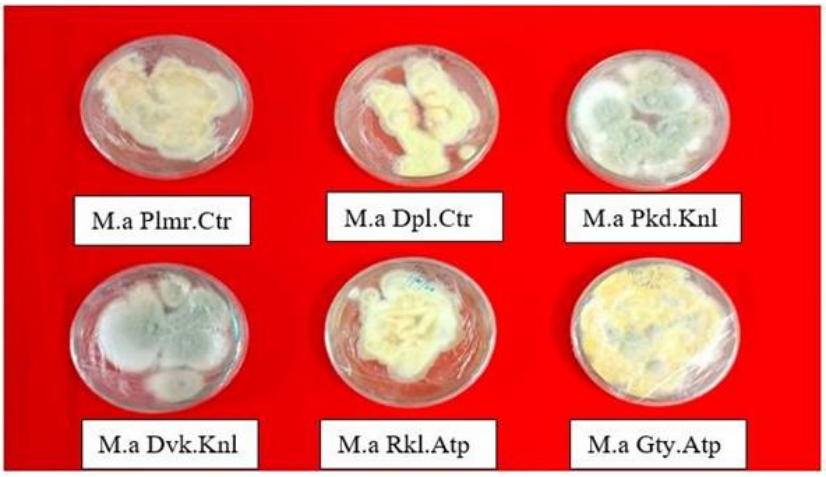

Fig. 2. Isolates of *Metarhizium anisopliae*

Table 1. Crops surveyed and the incidence of *Beauveria bassiana* and *Metarhizium anisoplaie* in different groundnut growing regions in

S. no	District	Village	Location	Crop	Cadavers noticed	Mean no. of cadavers/ sq.m
1.	Kurnool	Chakaralla	15.3889° N, 77.5562° E	Groundnut	*Spodoptera litura, Aproarema modicella.*	3.2
		Pothiralla	15.3964° N, 77.5008° E	Groundnut	*Spodoptera litura*	2.4
		Karivemula	15.5946° N, 77.6004° E	Groundnut,Cotton	*Helicoverpa armigera*	2.2
		Kapatralla	15.5936°N, 77.6316° E	Groundnut	Nil	
		Thondavada	13.5935° N, 79.3423° E	Groundnut	*Spodoptera litura*	2.2
2.	Anantapuramu	Ubicherla	15.1851° N, 77.6894° E	Groundnut	*Spodoptera litura*	1.4
		Reddipalle	14.0927° N, 79.2278° E	Groundnut	Nil	
		Rekulakunta	14.6938° N, 77.6695° E	Groundnut	*Spodoptera litura*	1.0
		Dandapalle	13.2392° N, 78.6966° E	Groundnut	*Aproarema modicella*	3.6
		Gangavaram	16.7339° N, 82.0475° E	Groundnut, cabbage	*Helicoverpa armigera*	2.4
3.	Chittoor	Gundlapalle	13.4477° N, 79.0363° E	Groundnut	*Spodoptera litura*	2.7
		Jagamarla	13.2215° N, 78.8248° E	Groundnut	Nil	
		Utukur	14.4538° N, 78.8123° E	Groundnut, Blackgram	*Spodoptera litura*	0.2
		Thadigotla	14.4950° N, 78.7639° E	Groundnut	Nil	
4.	Kadapa	Bhumayapal Le	14.6921° N, 78.7306° E	Groundnut, Maize	Nil	
		Miduturu	15.7709° N, 78.2889° E	Groundnut	Nil	

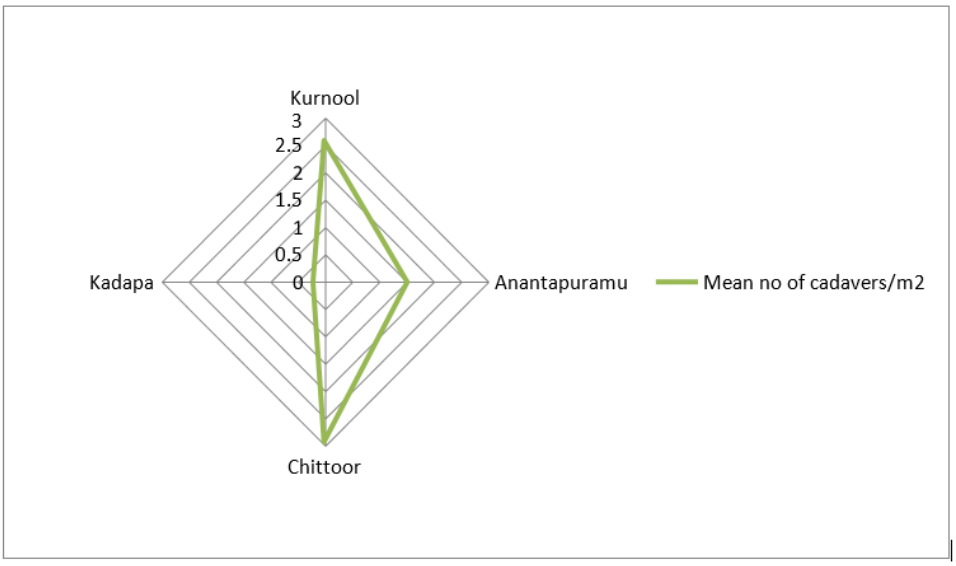

Fig. 3. Graphical representation of incidence of *Beauveria bassiana and Metarhizium anisopliae* in Rayalaseema zone of Andhra Pradesh

4. CONCLUSIONS

The present study reveals significant variations in the density of Entomopathogenic fungal (EPF) infected lepidopteran larvae across different districts. Chittoor district shows the highest density with 3.6 infected larvae per square meter, followed by Kurnool with 3.2 infected larvae per square meter. In contrast, Kadapa district has a considerably lower incidence of 0.2 infected larvae per square meter.These findings indicate that EPF can be a potent biological control agent for lepidopteran larvae in agricultural settings, particularly in groundnut and maize crops. The substantial presence of EPF in Chittoor and Kurnool suggests favorable conditions for the fungi, which can be harnessed for pest control. Farmers in these regions can utilize EPF either as a standalone solution or integrate it into Integrated Pest Management (IPM) strategies, reducing reliance on chemical pesticides. In Kadapa, where EPF incidence is low, efforts could be made to enhance EPF establishment and spread. Overall, incorporating EPF into pest management practices offers a sustainable, environmentally friendly approach to controlling lepidopteran larvae, promoting healthier agroecosystems and reducing chemical pesticide usage.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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